Over-diagnosis of rotavirus infection in infants due to detection of vaccine virus.

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ABSTRACT

Accurate rotavirus diagnosis is important for clinical management and monitoring active disease and vaccine effectiveness. Between 2016–2018, rotavirus-positive results in our laboratory were from vaccine virus shedding in 71/152 (46.7%) infants with a request for rotavirus testing. Routine diagnostic testing of infants should ideally distinguish vaccine from wild-type virus.

Keywords: rotavirus, vaccine, shedding, diagnosis

Where implemented, rotavirus vaccines have had a significant positive impact upon rotavirus-related healthcare use [1, 2]. In these settings, monitoring is required to ensure infection is accurately diagnosed and to allow reliable surveillance of vaccine effectiveness. Vaccination can however confound diagnosis of rotavirus infection, particularly in vaccinated infants and when using sensitive nucleic acid amplification tests (NAATs). This is because both the RotaTeq RV5 (Merck & Co.) and Rotarix RV1 (GSK Biologicals) rotavirus vaccines are live-attenuated vaccines that replicate in the gut, leading to vaccine virus being shed in the stools of vaccinated individuals. Pre-licensure studies reported RV5 vaccine virus shedding by virus culture in 13% of subjects' stools by day-7 following their first vaccine dose [3], while RV1 vaccine virus was detected in 76–80% of recipients after their first dose when using more sensitive ELISA-based antigen assays [4]. A post-implementation study using reverse transcriptase polymerase chain reaction (RT-PCR) assays reported 80–90% of vaccinated subjects shedding either RV5 or RV1 vaccine virus within 1–4 weeks after any vaccine dose [5].

In our community-based birth cohort study of 158 healthy infants conducted in Brisbane (the capital city of the state of Queensland, Australia), we employed weekly-collected stool samples to comprehensively examine the issue of rotavirus vaccine virus shedding in vaccinated infants when RV5 was part of the state-wide immunisation program [6]. We also observed that detection of RV5 nucleic acid following vaccination was frequent (47–87% of infants after each vaccine dose) and importantly we reported more prolonged (shedding up to 14-weeks duration after the third dose) than documented previously in clinical trials and post-licensure studies [3, 5]. These studies highlighted the importance of examining the influence vaccine strain shedding may have on clinical diagnosis and rotavirus surveillance data. Our interest in the potential for rotavirus positive results caused by vaccine shedding was also prompted by local pediatric infectious disease clinicians

expressing clinical concerns over the specificity of stool rotavirus results in infants following the transition from antigen-based to PCR assay testing by our local diagnostic laboratory in 2014.

METHODS

In this study, we used RT-PCR to distinguish vaccine from wild-type virus in stool samples submitted for routine rotavirus investigation and testing positive for rotavirus by RT-PCR at Pathology Queensland's Central Laboratory in Brisbane between November 2016 and March 2018. This period was particularly useful for conducting this study as up until July 2017 Queensland had used the RV5 vaccine (sample Bank 1) in its state-wide immunisation program before switching to the RV1 vaccine (sample Bank 2) [7]. Rotavirus vaccination information for the individuals whose specimens were tested for this study were not available to us. Clinical data were also not available, however given the request for rotavirus testing it is reasonably assumed all patients had diarrhea.

Overall, 465 rotavirus-positive samples were retested using specific assays for RV1 and RV5 viruses. Bank 1 comprised rotavirus-positive samples (n=65) collected from November 2016 to July 2017, during which time the RV5 vaccine was used in Queensland. The samples were from 32 females and 33 males, aged 1-week to 89-years (median 32.4-years, interquartile range [IQR], 1.4–64.7 years); 16 (24.6%) were infants aged <1-year. Bank 2 comprised rotavirus-positive samples (n=400) collected between October 2017 and March 2018, during which time the RV1 vaccine was used in Queensland. The samples were from 400 patients including 179 females and 221 males, aged 5-days to 98-years (median 10.0-years [IQR, 0.3–59.4 years]); 136 (34.0%) were aged <1-year.

Briefly, the pan-rotavirus RT-PCR used by the Diagnostic Laboratory is a laboratory-developed PCR assay based on the method described by Pang et al., 2011 [8]. The RV1 and RV5 RT-PCR assays

employed to test all 465 rotavirus positive samples followed methods described previously (Gautam et al., 2014 [9]); the RV5 method was further adapted for use in our research laboratory during the earlier community-based birth cohort study [6]. Norovirus and adenovirus PCR results were also available for all samples from the routine PCR testing conducted by the Diagnostic Laboratory.

The Children's Health Queensland Human Research Ethics Committee (HREC/16/QRCH/299) approved the study.

RESULTS

The results of the RV1 and RV5 RT-PCR testing are summarized in the Figure. The results for all PCR tests, including adenoviruses and noroviruses, for each individual patient are detailed in the Supplementary Dataset, Tables 1 and 2. Of the 65 rotavirus-PCR positive samples from Bank 1, seven (10.8%) samples were RV5 positive, and all were from infants aged <1-year old (Figure). Sixteen patients in Bank 1 were aged <1-year, and thus RV5 comprised 43.8% (7/16) of all detections in this age group. No RV5 was detected in any patient older than 1-year of age in Bank 1, and none of the Bank 1 samples were positive for the RV1 vaccine strain. For Bank 2, 64/400 (16.0%) samples were positive for the RV1 vaccine strain and were from infants aged 6–28 weeks, except for one child aged

3-years and a man aged in his fifties. Two infants <1-year of age from Bank 2 were positive for the RV5 vaccine strain. For Bank 2, RV1 comprised 45.6% (62/136) and RV5 1.5% (2/136) of all detections in those <1-year of age. Vaccine virus detection in infants aged <1-year was not significantly different between Bank 1 and 2 samples (relative risk = 0.93, 95% confidence interval 0.52, 1.67).

In total, 92/465 (19.8%) rotavirus positive samples were also positive for enteric adenovirus 40/41 and/or norovirus, with adenovirus 40/41 accounting for most (70/92; 76.1%) co-detections

(Supplementary Dataset, Tables 1 and 2). Co-detections were observed for 12/65 (18.5%) samples from Bank 1 and 80/400 (20.0%) from Bank 2. For Bank 1, these comprised 2/7 (28.6%) RV5-positive samples (both adenovirus 40/41 co-detections) and 10/58 (17.2%) for wild-type rotavirus samples (6 adenovirus 40/41, 3 norovirus and 1 adenovirus/norovirus co-detection). For Bank 2, co-detections comprised 11/64 (17.1%) RV1-positive samples (8 adenovirus 40/41 and 3 norovirus) and 67/334 (20.1%) wild-type samples (48 adenovirus 40/41, 16 norovirus and 3 adenovirus/norovirus co-detections). For the two samples from Bank 2 that were RV5 positive, both were co-detected with adenovirus 40/41.

DISCUSSION

These data highlight the potential for sensitive NAAT methods to over-diagnose rotavirus infection in infants due to shedding of either RV1 or RV5 vaccine virus [10]. Positive results caused by vaccine shedding in young infants may adversely affect clinical management by delaying serious alternative diagnoses and their treatment, such as urinary tract infections or intra-abdominal sepsis and prolonging unnecessary infection control precautions [11]. Furthermore, misclassifying rotavirus positive cases can lead to both vaccine program effectiveness being under-estimated and healthcare-associated rotavirus infection being over-estimated. Notably, in this regard almost half (46.7%) of infants aged <1-year provided a positive result for rotavirus infection due to shedding of vaccine virus. These findings are consistent with the RV5 data observed previously in our community birth cohort study [6], reports of RV1 vaccine strain shedding in stools for several weeks after vaccination [12], and recent Australian studies showing that RV5 vaccine viruses comprised 71.9% of detections in children aged <32-weeks in South Australia [13] and RV1 comprised 71% of detections in infants aged ≤6-months in New South Wales [14].

Potential limitations of this study are that specimen banks were samples of convenience available from a local pathology provider and the availability of samples from the earlier RV5 vaccine period (Bank 1) were limited by the provider's sample storage capacity (hence the lower numbers for Bank 1). In addition, we relied upon physician testing behaviour, and there was also no access to clinical or vaccination data. Nevertheless, these data emphasize the need for rotavirus diagnostic and screening methods to distinguish vaccine from wild-type virus when testing recently vaccinated infants using NAAT platforms [10]. In circumstances where vaccine virus is co-detected in stools with recognized enteric pathogens, such as norovirus, adenovirus 40/41 or non-typhoidal *Salmonella,* the latter organisms are more likely to be the cause of the child's diarrheal symptoms [6]. However, in settings where available tests cannot distinguish vaccine and wild-type virus, then a suitable comment should accompany the test result indicating that detection of rotavirus RNA in infants is not necessarily an indication of infection, but may be due to vaccine shedding.

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CONFLICT OF INTEREST

DW reports research funding from SpeeDx Pty Ltd. KG participated in a rotavirus strain outbreak advisory board for GlaxoSmithKline, Rixensart, Belgium. All other authors have nothing to disclose.

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Figure 1 legend:

Results of the RV1 and RV5 RT-PCR testing

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